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Original Research Article

Heat shock protein 60 and chromatin assembly factor-1 mRNA levels in hepatitis C virus-related hepatocellular carcinoma and clinical significance

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the third cause of cancer-related death worldwide. Heat Shock protein 60 (HSP60), a mitochondrial chaperone, is overexpressed in diverse malignant cells. Chromatin Assembly Factor-1 (CAF-1), a histone chaperone, is down-regulated in quiescent non-proliferating human cells. We aimed to clarify the role of HSP60 and CAF-1 mRNA expression in diagnosis of HCC post-HCV infection.
Methods: HSP60 and CAF-1 mRNA levels in urine and blood were quantified by Taqman real-time PCR in 49 subjects; 25 cirrhotic with HCV-related HCC, 12 cirrhotic without HCC and 12 healthy controls.
Results: HSP60 and CAF-1 mRNA levels in urine and blood were significantly higher in HCC versus cirrhosis and controls, and in cirrhosis versus controls. Their levels in HCC were significantly increased by advancement of HCC BCLC staging system. HSP60 in urine had 85% sensitivity and 66% specificity at cut off 258354 RU and 85% sensitivity and 60 % specificity at cut off 37576 RU in blood for HCC diagnosis. CAF-1 in urine had 81% sensitivity and 66% specificity at cut off 137756 RU and 77% sensitivity and 64% specificity at cut off 49726 RU in blood for HCC diagnosis. HSP60/CAF-1 sensitivity and specificity in urine and blood were better than either marker alone, with better results in urine (91% and 73%, respectively) than blood (88% and 66%, respectively).
Conclusions: HSP60 and CAF-1 in urine and blood may be useful HCC diagnostic markers that were correlated with advancement of HCC with better combined marker sensitivity and specificity than either marker alone especially for urine.

Keywords: CAF-1, Gene expression, HCC, HSP60, Taqman real-time PCR, Urine

INTRODUCTION

Worldwide, hepatocellular carcinoma (HCC) is graded sixth in incidence and third in mortality among all cancers.[1] Chronic viral hepatitis B and C account for 80-90% of all HCC cases being major risk factors.[2] In Egypt, HCV infection causes up to 40-50% of HCC cases. The overall 5-year survival rate of HCC is 5-9% from the time of diagnosis and 69% for patients undergoing hepatectomy, especially for early detected tumor which is a single nodule below 2 cm. The dismal prognosis is largely due to late detection.[3] Early detection of HCC by

serum alpha fetoprotein (AFP) is limited by its low sensitivity, so there is an urgent need for new high accurate and reliable non-invasive biomarkers with high accuracy and feasibility.[4]

Heat shock proteins (HSPs), a high conserved functional superfamily, play important roles in protein folding and translocation.[5] HSPs are stress proteins whose synthesis is exaggerated by proteotoxic stresses such as heat shock.[6] The superfamily members, based on their molecular weight, include HSP90, HSP70, HSP60, HSP40 and small HSPs including HSP27.[7] HSP60, a typical mitochondrial chaperone, is associated with progression of diverse cancers. However, its expression and significance in HCC are still largely obscure.[8]

Chromatin assembly factor-1 (CAF-1) is a highly conserved heterotrimeric histone chaperone consists of p48, p60 and p150 protein subunits and plays a critical role in DNA replication and repair, though regulation of chromatin assembly (deposition of histones H3 and H4, required for S-phase progression of the cell cycle, on newly synthesized DNA).[9,10] CAF-1 gene expression is massively down-regulated in quiescent cells and appears essential for human cell proliferation.[11] CAF-1 is overexpressed in many cancers.[12]

Extracellular nucleic acids, both DNA and mRNA, were found to exist in many biological media; serum, plasma, saliva, urine, semen, milk, bronchial lavage, and cell culture supernatants.[13] The detection of RNA tumor markers in urine has been reported as an emerging tool for noninvasive tumor diagnosis.[14]

Aim of the work was to clarify the role of HSP60 and CAF-1 mRNA gene expression in blood and urine in diagnosis of HCV-related HCC.

METHODS

The present study was a comparative observational study performed at the Hepatology, Gastroenterology and Infectious Diseases Department and the Molecular Biology Unit, Faculty of Medicine, Benha University during the period from January to November 2014. Approval of the study by the Ethical Scientific Committee of Benha Faculty of Medicine was obtained.

This study included 3 groups; Group I: included 25 HCC post-HCV patients diagnosed by two radiological methods with or without AFP higher than 200ng/ml. Group II: included 12 liver cirrhosis patients, diagnosed by clinical, laboratory and imaging methods. Group III: included 12 healthy controls subjects who were age and sex matched with patients.

Informed medical consents were obtained from subjects before enrollment in the study. Patients <18 years, malignancies other than HCC and co-infection with chronic hepatitis B were excluded. All patients and controls were subject to thorough history taking, complete clinical examination and routine laboratory investigations. Abdominal ultrasonography, abdominal spiral triphasic computed tomography (for HCC) and Barcelona Clinic Liver Cancer (BCLC) staging classification (for HCC) were done. Quantitation of HSP60 and CAF-1 mRNA by Taqman quantitative real-time PCR in blood and urine was performed for all subjects.

Sampling

A venous blood sample (~7ml) was taken from each subject of which 2ml were put into sterile vaccutainer tube containing ethylene diamine tetra-acetic acid (EDTA); one 1ml was used for CBC by automated hematology analyzer Sysmex XS-1000i (Sysmex, Japan), and the other 1ml was stored at –80ºC in nuclease-free sterile eppendorff tube for real-time PCR.[15] Another 1.8ml were withdrawn in a tube containing tri-sodium citrate solution (3.8%) in a ratio of 9:1 for determination of prothrombin time (PT) by Behring Fibrintimer II (Behring, Germany).[16]

The remaining part (~3ml) was left to clot and serum was separated for determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, total bilirubin, creatinine, alpha fetoprotein (AFP) by Microtech spectrophotometer (Vital Scientific, Netherlands), hepatitis markers including hepatitis B surface antigen (HBs-Ag) by enzyme immunoassay, HCV antibodies (HCV-Ab) by enzyme immunoassay and HCV-RNA quantitation by real-time PCR.[17-24]

A first void morning urine sample (3ml) was put in a sterile tube containing EDTA (EDTA has antinuclease action by ion depletion so deactivating metal-dependent enzymes), then centrifuged at 3000xg for 5min and the supernatant (1.5ml) was put in a nuclease-free sterile eppendorff tube then kept at -80ºC for further assessment.[25]

Determination of HCV-RNA viral load

QIAamp Viral RNA mini Kits were used for HCV- RNA extraction by Qiacube automated extraction (Qiagen, Germany). This was followed by absolute quantitative real-time PCR using artus HCV RG RT-PCR Kit (Qiagen, Germany) with the standard curve generated through 4 quantitation standards supplied on StepOne real-time PCR system (Applied Biosystems, USA). Result Calculation was performed according to the equation:

Result (IU/ml) = Result (IU/µl) x Elution Volume (µl) / Sample Volume (ml)

All steps were performed according to the manufacturers' instructions.

Quantitative real-time PCR for HSP60 and CAF-1 mRNA

Total RNA extraction from blood

From blood (200µl) and urine supernatant (1000µl) was done using PurelinkTM RNA mini kit, (Life Technologies) following the manufacturer´s instructions in addition to DNase digestion by Purelink RNase-free DNase set (Life Technologies). Eluted RNA (200µl) was collected and quantified immediately by Ultraviolet Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), then kept at -20ºC for further assessment.

Absorbance at 260nm should be >0.15. An absorbance of 1unit at 260nm corresponds to 40µg of RNA/ml. The ratio between the absorbance values at 260 and 280nm gives an estimate of RNA purity, pure RNA has a ratio of 1.9-2.3.[26]

Relative quantitation of respective gene mRNA by real-time PCR: on 2 steps

The 1st step: for conversion of RNA into cDNA using High-Capacity cDNA Reverse Transcription kit (Applied Biosystem, Foster City, USA). The master mix for reverse transcription (RT)/RNA sample contained 10X RT-PCR (2µl), 25X dNTP mix (0.8µl), 10X RT random primers (2µl), MultiScribeTM Reverse Transcriptase (1µl), RNase inhibitor (1µl) and nuclease-free water up to 20µl. The PCR mix for RT reaction of RNA into cDNA contained 2X RT master mix (10µl) and Extracted RNA (10µl). RT was performed in G-Storm Thermal Cycler (United Kingdom) with cycling conditions; 25ºC for 10min, 37ºC for 120min and 85ºC for 5min)

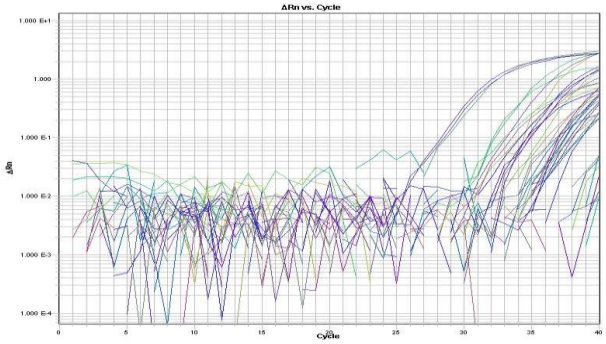
The 2nd step: was for quantitation of HSP60 and CAF-1 mRNA in an ABI 7900HT real-time PCR (Applied Biosystem, Foster City, USA). The reaction components were done in MicroAmp® 96 Well Optical Reaction Plate with Barcode, code 128). cDNA was diluted 1: 20 (2µl cDNA + 40µl nuclease free water). Singleplex reactions were done. This step was performed using Taqman® Universal Master Mix II, No UNG kit (Applied Biosystem, Foster City, USA).

The assays for human HSP60 and CAF-1 as target genes and human GAPDH as an endogenous control housekeeping gene were used. Primers and probes were provided as ready to use Taqman gene expression assays. Each assay contains a mix of forward and reverse primers and a reporter dye labeled Taqman® minor groove binder probe.

The probe was FAM-labeled for CAF-1 (Hs04189672_g1) and HSP60 (Hs01036753_g1) but VIC-labeled for GAPDH (NM_002046.3). All assays were purchased from Applied Biosystem, Foster City, USA. The reaction mix contained Taqman RT-PCR Universal Master Mix, No Amperase® UNG (10µl), Taqman gene expression assay mix 20X (1µl) and up to 20µl diluted cDNA. Thermal conditions were holding (10min at 95ºC), denaturation (15sec at 95ºC) and annealing/extension (1min at 60ºC).

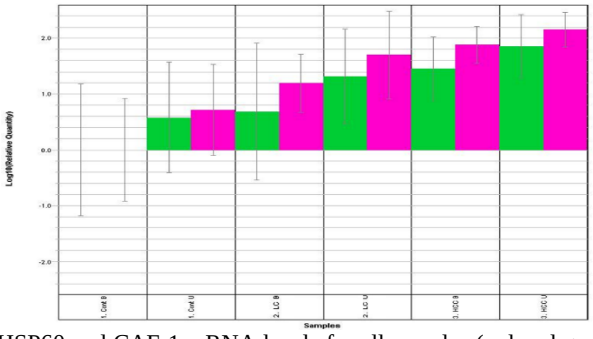
Data analysis

According to the RQ manager program SDS software (ABI 7900HT), the data were produced as sigmoid shaped amplification plots in which the cycle number was plotted against fluorescence (on linear scale), and the cycle number was plotted against the magnitude of signal in the amplification plot (Figure 1). The cycle threshold (CT) serves as a tool for calculation of the starting template amount in each sample.



Amplification plot curves for HSP60 and CAF-1 mRNA expression in the studied groups (urine and blood samples); the cycle number was plotted against the magnitude of signal.

Figure 1: Amplification plot curves for target genes mRNA expression in the studied groups (urine and blood samples).



HSP60 and CAF-1 mRNA levels for all samples (red and green bars respectively). The levels are represented in log10 values for relative quantitation (RQ). The level of the control samples appear 0 in the graph because the log10 for 1 is 0. The RQ for HSP60 and CAF-1 are normalized against the RQ of GAPDH (endogenous control), so the expression level of the endogenous control is 0 (no bars for GAPDH) (B blood, U: urine).

Figure 2: HSP60 and CAF-1 mRNA levels for all samples (red and green bars respectively).

The control samples were used as calibrators so the expression levels were set to 1. The gene expression levels (Figure 2) were plotted as log10 values so the

expression level of calibrators appear as 0 in the graph (log10 of 1 is 0). The relative quantities of the CAF-1 and HSP60 mRNA were normalized against that of GAPDH (endogenous control), so fold expression changes were calculated using the equation $2^{-\Delta\Delta CT}$.[27]

Statistical tests

Statistical analysis of results was done using SPSS computer package version 10. Quantitative data were expressed as mean ± standard deviation (mean±SD). Qualitative data were presented as frequencies and percentage. ANOVA with post-hoc test was used to test the significance of difference between the mean value of more than two groups and Chi-square test was used for comparison of categorical variables. Correlations between data were performed using Pearson correlation tests. Receiver Operator Characteristics (ROC) curve was used to determine cutoff value of HSP60 and CAF-1 with optimum sensitivity and specificity in diagnosis of HCC. $p<0.05$ was significant.

RESULTS

The present study showed non-significant age and sex differences in HCC versus liver cirrhosis and controls ($p>0.05$ for both). Concerning Child's classification; Child class A accounted for 8% of HCC, 64% for class B and 28% for class C (Table 1). There were also significantly increased platelets, albumin, bilirubin, PT, ALP and AFP in HCC compared to cirrhosis and controls and significantly increased platelets and albumin in cirrhosis versus controls. Moreover, HSP60 and CAF-1 mRNA level Relative Unit (RU) and fold changes in urine and blood were significantly higher in HCC than cirrhosis and controls ($p<0.001$) (Table 2, Figure 3).

Table 1: Clinical data of the studied groups.

Variable		HCC (n=25)	Liver cirrhosis (n=12)	Controls (n=12)	P value
Age	mean±SD[a]	55±4.7	47±6.3	44±4.7	>0.05
Sex (♂/♀)	n (%)[b]	18 (72)/7 (28)	8 (66.7)/4(33.3)	8 (66.7)/4 (33.3)	>0.05
	ratio	2.5:1	2:1	2:1	-
Child score A/B/C	n (%)[b]	2 (8)/16 (64)/7 (28)	6 (50)/5 (41.7)/1 (8.3)		

[a]: Anova test, [b] :Fisher's exact test

There was a significant positive correlation between HSP60 and CAF-1 mRNA levels in blood and urine on one hand and focal lesion characters (size and number) on the other hand, but no correlation was detected with AFP (Table 3).

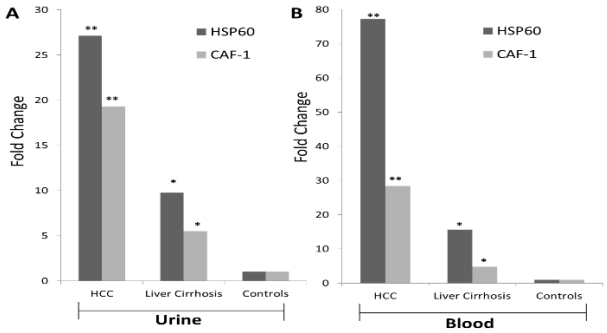
Table 2: Laboratory characters of the studied groups.

Variable	HCC (n=25)	Liver cirrhosis (n=12)	Controls (n=12)
	Mean±SD		
Hemoglobin (g/dl)	10.1±0.9[b]	12.3±0.8	13.8±0.8
Platelets ($\times10^3$ c/mm^3)	126.1±38.5[a,b]	183.7±59.5[a]	280.7±47.9
Aspartate aminotransferase (IU/L)	37.2±10.8	36.3±8.4	33.3±4.5
Alanine aminotransferase (IU/L)	38.3±11	35.8±7.8	34.7±5.8
Serum albumin (g/dl)	2.4±17[a,b]	3.3±4.7[a]	4.3±3.1
Serum total bilirubin (mg/dl)	4.2±2.7[a,b]	1.8±0.6	1.1±0.3
Prothrombin time (seconds)	15.8±1.9[a,b]	13.3±1.1	12.6±0.5
Serum creatinine (mg/dl)	1.9±0.6	1.4±0.4	1.3±0.2
Alkaline phosphatase (IU/L)	231.9±70.2[a,b]	127.6±24.8	101.2±16.5
Alpha fetoprotein (ng/ml)	550.5±578.7[a,b]	10.4±9.3	1.4±1.5
HCV-RNA (IU/ml)	413433.2±384012.9	139558.4±173773.7	-
HSP60 (RU) in urine	278870.8±12791.6[c,d]	100396.8±6990.3[c]	10281.3±1364.2
HSP60 (RU) in blood	153962.6±5533.8[c,d]	31088±3370.5[c]	1994.6±424.2
CAF-1 (RU) in urine	147295.1±9004.2[c,d]	42041±8509.9[c]	7633.3±644.1
CAF-1 (RU) in blood	57610.4±5184.3[c,d]	9766.6±952.5[c]	2029±417.6

Anova test was used, HCV-RNA: ribonucleic acid, RU: relative unit, [a]: $p<0.05$ versus control, [b]: $p<0.05$ versus liver cirrhosis, [c]: $p<0.01$ versus controls, [d]: $p<0.01$ versus liver cirrhosis

Table 3: Correlation between HSP60 and CAF-1 mRNA and both AFP and focal lesion characters (size and number).

Variable	Focal lesions size		Focal lesions number		AFP	
	r	p	r	P	R	p
HSP60 in urine	0.69	<0.05*	0.70	<0.05*	0.25	>0.05
HSP60 in blood	0.63	<0.05*	0.72	<0.05*	0.15	>0.05
CAF-1 in urine	0.67	<0.05*	0.63	<0.05*	0.25	>0.05
CAF-1 in blood	0.61	<0.05*	0.67	<0.05*	0.14	>0.05



Fold change of HSP60 and CAF-1 in both urine (A) and blood (B) were highly statistically significantly increased in HCC versus liver cirrhosis and controls and in liver cirrhosis versus controls. Urine HSP60 fold increase in HCC and cirrhosis versus controls were; 27.12 and 9.76; however in blood it was 77.2 and 15.6, respectively. Urine CAF-1 fold increase in HCC and cirrhosis versus controls were 19.3 and 5.51; however in blood it was 28.4 and 4.8, respectively. **: high significant versus liver cirrhosis and controls ($p<0.01$), *: high significant versus controls.

Figure 3: Fold change of HSP60 and CAF-1 mRNA expression in urine and blood in the studied groups.

HSP60 and CAF-1 mRNA levels in both blood and urine were significantly increased by advancement of BCLC system stages of HCC (Table 4).

Urine HSP60 at cutoff level 258354 relative unit (RU) had 85% sensitivity and 66% specificity for HCC diagnosis with 77% diagnostic accuracy and 0.71 area under the curve (AUC). Also, HSP60 in blood was able to diagnose HCC with 85% sensitivity and 60 % specificity at cutoff level 37576 RU with 75% diagnostic accuracy and 0.68 AUC. Regarding urinary CAF-1, its sensitivity and specificity for diagnosis of HCC were 81% and 66% at cutoff 137756 RU with 73% diagnostic accuracy and 0.70 AUC. Moreover, CAF-1 in blood at cutoff 49726 RU had sensitivity and specificity of 77%, 64% respectively with 72% diagnostic accuracy and 0.66 AUC. The combined urine HSP60/CAF-1 sensitivity and specificity were 91% and 73%, respectively with 78% accuracy and 0.75 AUC. The combined blood HSP60/CAF-1 sensitivity and specificity were 88% and 66%, respectively with 77% accuracy and 0.70 AUC (Table 5, Figure 4).

Table 4: HSP60 and CAF-1 mRNA level in different BCLC staging system of HCC.

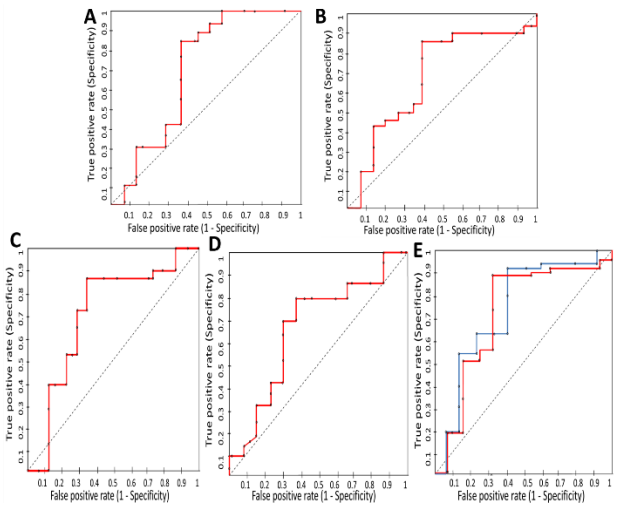
Variables	BCLC staging system				p
	Stage A (n=7)	Stage B (n=6)	Stage C (n=5)	Stage D (n=7)	
HSP60 (RU) in urine	251351.3±87.4	270699.4±18.9	288733±93.3	293699.7±72.7	<0.05*
HSP60 (RU) in blood	140785.6±45.04	153601.4±39.1	169355.5±57.7	173607.5±65.9	<0.05*
CAF-1 (RU) in urine	136012.6±62.02	148880±87.7	160389.5±14.5	163292.1±10.5	<0.05*
CAF-1 (RU) in blood	44900.50±27.1	56679.6±63.6	69996±53.6	72163.3±64.7	<0.05*

Values expressed as mean±SD, Anova test was used, *: significant

Table 5: Diagnostic performance of HSP60 and CAF-1 mRNA in blood and urine for HCC diagnosis.

Variable	Cutoff level	Sensitivity %	Specificity %	Accuracy %	NPV %	PPV%	AUC
HSP60 in urine	258354 RU	85	66	77	76	78	0.71
HSP60 in blood	37576 RU	85	60	75	75	75	0.68
CAF-1 in urine	137756 RU	81	66	73	75	79	0.70
CAF-1 in blood	49726 RU	77	64	72	64	77	0.66
HSP60/CAF-1 in urine		91	73	78	77	80	0.75
HSP60/CAF-1 in blood		88	66	77	73	78	0.70

RU: relative unit, NPV: negative predictive value, PPV: positive predictive value, AUC: area under the curve.



ROC curves of HSP60 and CAF-1 mRNA in urine and blood for diagnosis of HCC; A: HSP60 mRNA in urine, B: HSP60 mRNA in blood, C: CAF-1 mRNA in urine, D: CAF-1 mRNA in blood, E: combined HSP60/CAF-1 mRNA in urine and blood

Figure 4: ROC curves of HSP60 and CAF-1 mRNA in urine and blood for diagnosis of HCC.

DISCUSSION

The most common primary liver cancer is HCC, around 90%.[27] Worldwide, HCC is the fifth most common cancer in males, and ninth in females.[29] In Egypt, HCC is the second most common cancer in males and sixth in females.[30] Abdel-Atti reported that HCC is present in 21% of Egyptian cirrhotic patients.[31] The multistep process of liver carcinogenesis involves various genetic and phenotypic alterations.[32] The current study aimed to identify the role of HSP60 and CAF-1 in urine and blood mRNA as non-invasive biomarkers for HCC diagnosis. In the current study, HCC patients were older (55±4.7 years) than cirrhotics patients and controls but without statistical significance ($p>0.05$). This result agreed with Abdelaziz et al. who reported that, the mean age of HCC patients was 56.5±7.7 years. HCC was more frequent in males in the present study with male to female ratio (2.5:1).[33] This male predominance came in agreement with Keng et al. who reported the same ratio for HCC universal estimated male/female ratio.[34] The current study revealed highly significantly up-regulated HSP60 mRNA in both urine and blood of HCC versus liver cirrhosis and control groups ($p<0.001$ for both). This finding agreed with Abdalla and Haj-Ahmad who documented that urine HSP60 was significantly over-expressed in HCC compared to chronic HCV and controls.[11] Also Beere documented that blood HSP60 seems to have prognostic value in HCC.[35] Using proteomics approach, Kuramitsu and Nakamura demonstrated that HSP60 was increased in HCV-related HCC specimens compared to non-cancerous liver tissues.[36] As an explanation, HSP60 was the most critical and sensitive redox chaperone required for hepatocyte growth factor (HGF)-stimulated extracellular signal-regulated kinases (ERK) phosphorylation, cell migration and tumor progression in HepG2 and HCC340 cell lines. HSP60 was a promising therapeutic target for preventing HGF-induced HCC progression mediated by reactive oxygen species.[37] On the contrary, Zhang et al reported down-regulated HSP60 expression in HCC tissue compared to peritumor tissue. They reported that HSP60 exerted a tumor suppressor function and that its overexpression induced HCC cell differentiation with good prognosis.[8] A previous study by Lim et al. reported non-significant HSP60 difference between tumor and non-tumor HBV-related HCC specimens by immunohistochemistry and dot-immunoblotting. [38] The present study showed a highly significant expressed CAF-1 mRNA levels (urine and blood) in HCC cases compared to cirrhosis and controls ($p<0.001$). This result agreed with Abdalla and Haj-Ahmad who found significantly higher CAF-1 in urine of HCC versus chronic HCV and controls.[11] On the same hand, Li et al., found up-regulated CAF-1 expression in HCC.[39] In addition, Xu et al. reported increased CAF-1 in HCC tumor tissues versus the surrounding non-tumor tissues by immunohistochemistry, mRNA and protein expression in HCC patients and cell lines, and they concluded that enhanced CAF-1 expression promoted tumorigenesis and progression by promoting HCC cell proliferation through the dysregulated DNA damage checkpoints or inappropriate assembling of nucleosomes affecting DNA replication.[40]

The current study observed increased urine HSP60 and CAF-1 in cirrhotic group compared to controls ($p<0.01$). This result agreed with Abdalla and Haj-Ahmad, who explained the increased CAF-1 in chronic HCV by the role of CAF-1 in stimulating the proliferation of quiescent hepatic stellate cells and its role in the development of fibrosis, cirrhosis and HCC on top. They explained the up-regulated HSP60 in chronic HCV by its anti-apoptotic effect rather than being death-promoting for HCC.[11]

Moreover, in HCC group, we found significant positive correlation between HSP60 and CAF-1 (urine and blood) and tumor characters (size, number) and BCLC staging system ($p<0.05$), but no significant correlation with AFP could be detected. These finding were in accordance with Li et al and Joo et al, the former documented that CAF-1 over-expression indicated poor HCC prognosis and the later found a strong positive correlation between HSPs and clinic-pathological grading of HCC.[39,41] On the same hand, Lianos et al. reported that HSPs over-expression was related to poor prognosis, therapeutic resistance and poor survival of cancer patients.[42] On the other hand, Zhang et al. reported that HSP60 expression was significantly correlated with serum AFP and its high expression in cancer compared to pericancer tissue was associated with better overall survival.[8] Moreover, Lim et al. found that HSP60 had no relation with histopathological grading and not affecting the survival in HBV-related HCC.[38] In agreement with our study; Xu et al. observed a significant positive correlation between CAF-1 expression and both tumor number and size but

no significant correlation with AFP.[40] In the present study, HSP60 mRNA in urine at cutoff 258354 RU had 85%, sensitivity and 66% specificity for HCC diagnosis, a result agreed with Abdalla and Haj-Ahmad, who found 83% sensitivity and 43% specificity for HSP60 in urine for diagnosis of HCC.[11] Also, HSP60 in blood was able to diagnosis HCC with 85% sensitivity and 60% specificity at cutoff 37576 RU with no supported published data. Regarding urine CAF-1, its sensitivity and specificity for diagnosis of HCC in the current study were 81% and 66% respectively at cutoff 137756 RU. Abdalla and Haj-Ahmad reported that urine CAF-1 had sensitivity 66% and specificity 90% for diagnosis of HCC.[11] Moreover, CAF-1 in blood at cutoff level 49726 RU had 64% specificity and 77% sensitivity for diagnosis of HCC with no supported published data. The combined blood HSP60/CAF-1 had 88% sensitivity and 66% specificity. The combined urine HSP60/CAF-1 sensitivity was 91% and specificity 73%; however, Abdalla and Haj-Ahmad found 61% sensitivity and 91% specificity. [11] Present results concerning both marker sensitivity and specificity in urine were better than that of blood. Cell-free nucleic acids, both DNA and mRNA, may reach urine through transport of circulating nucleic acids from blood into urine.[43] Moreover, the use of 1st void morning urine sample is quite common in diagnostic studies because of higher nucleic acids content.[44] So urine allows an easy obtained, non-invasive sample for real-time quantitative PCR-based markers (HSP60 and CAF-1).

CONCLUSION

HSP60 and CAF-1 mRNA levels in both urine and blood were increased in HCC patients versus cirrhosis and controls, so they may be used as useful diagnostic markers for HCC and as urine is an easy, non-invasive sample, it may be used in screening of patients at high risk.

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